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Human parvovirus B19 infection of monocytic cell line U937 and antibody-dependent enhancement

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Abstract

Human parvovirus B19 (B19) infects human erythroid lineage cells. Accumulating evidence also shows that B19 is detectable in nonerythroid lineage cells in vivo, but the mechanism of infection is still not clear. In this study, we explored the mode of B19 infection of human monocytic cell line U937. An in vitro infection study demonstrated B19 binding of U937 and slow replication of B19-DNA with B19-NS1 mRNA transcription. B19-DNA replication in U937 was accompanied by undetectable level of B19-VP1 mRNA transcription, indicating that B19 infection of U937 cells may be abortive. Levels of B19-DNA and B19-NS1 mRNA transcription increased in the presence of anti-B19 IgG antibodies, but this effect decreased in the presence of anti-Fc receptor antibodies, showing antibody-dependent enhancement by B19 infection. Antibody-dependent enhancement also caused the increased production of TNFα in U937. This study is the first to suggest B19 infection of nonerythroid lineage cells with antibody-dependent enhancement.

Keywords: Parvovirus B19; Antibody-dependent enhancement; TNFα

Introduction

Human parvovirus B19 (B19) is an erythrovirus that infects human erythroid lineage cells and causes hemolytic anemia or hydrops fetalis (Brown and Young, 1997). The B19 genome includes major open reading frames coding for nonstructural protein NS1 and structural proteins VP1 and VP2 (Shade et al., 1986). The cellular receptor for B19 infection has been identified as P antigen that is a glycolipid expressed on erythroid lineage cells (Brown et al., 1993). However, B19 is often detectable in nonerythroid cells in vivo (Anderson et al., 1985; Isumi et al., 1999; Ray et al., 2001; Takahashi et al., 1998; Weigel-Kelley et al., 2001). B19 infection may also cause autoantibody production, such as rheumatoid factors, anti-nuclear antibody, anti-DNA antibody or anti-phospholipid antibody and autoimmune disease-like signs (Kerr, 2000; Lehmann et al., 2003; Ochiai et al., 1988). We have recently described the presence of B19-DNA, mRNA and protein (VP1) in macrophages, follicular dendritic cells, T cells and B cells in synovial tissues from patients with rheumatoid arthritis. VP1 has been detected in U937 monocytic cell line when coincubated with rheumatoid synovial cells in vitro (Takahashi et al., 1998). These findings are unexplainable solely by B19 infection of erythroid cells. Hemophagocytic syndrome or necrotizing lymphadenitis, where proinflammatory cytokines such as tumor necrosis factor alpha (TNFα) from phagocytes are responsible for the pathogenesis, is accompanied by B19 infection (Johnson et al., 2003; Larroche et al., 2002; Lay et al., 1997), and we have shown that transfection of the B19-NS1 gene into U937 leads to an increased production of TNFα (Fu et al., 2002). Verification of B19 infection in nonerythroid cells, especially those that function as phagocytes, may provide crucial information on clinical problems of B19 infection.

In this study, we investigated the mode of B19 infection of nonerythroid cells by assessing in vitro B19 infection of U937 monocytic cell line that is supposed to be the experimental

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model system of B19 infection of antigen-presenting cells (APC) and also explored whether antibody-dependent enhancement (ADE) may be involved in B19 infection of U937 because anti-viral neutralizing antibodies which bind to the Fc receptor on phagocytes often facilitate viral infection of their target cells, resulting in persistent infection in vivo (Cancel Tirado and Yoon, 2003; Halstead, 1988; Halstead and O'Rourke, 1977; Hawkes, 1964).

Results

B19-DNA was detected in PBMC from patients with acute B19 infection

To explore the possibility of B19 infection of nonerythroid lineage cells in vivo, we first examined the presence of B19-DNA in PBMC in patients with acute B19 infection. B19-DNA was detected at 15,864 ± 1592 copies/μg DNA in isolated PBMC and at 3492 ± 122 copies/μg DNA even after the treatment with pronase. Nuclear extract from the same PBMC contained 2926 ± 204 copies/μg DNA of B19-DNA. The difference between the B19-DNA copy number in pronase-treated PBMC and in the nuclear extract showed no statistical significance. Copy numbers of B19-DNA from uninfected normal subjects were below 10 copies. Copy numbers of B19-DNA in PBMC from normal subjects were below 200 copies at 10 min after B19 inoculation in vitro (data not shown).

B19 shows abortive infection in U937 monocytic cell line

To know if nonerythroid cells participate in B19 infection in vitro, we examined the replication of B19-DNA in U937 monocytic cell line as the model of B19 infection to nonerythroid cells. P antigen, the known B19 receptor, was not detectable on the surface of U937 (data not shown). Initial adsorption of B19 to U937 was observed at Day 0 (43,300 ± 2600 copies). The copy number of B19-DNA decreased to 7100 ± 600 copies at Day 4 (Fig. 1A), and it tended to increase from Day 4. The copy number of B19-DNA at Day 12 was 14,500 ± 1700, which was a statistically significant increase compared with the copy number at Day 4 ($P < 0.01$). Next, we evaluated the transcription of B19-mRNA in U937 cells. We detected 2031 ± 164 copies/(10^{10} copies of GAPDH mRNA) of B19-NS1 mRNA at Day 6 by using quantitative RT-PCR but was not detected without reverse transcription (Fig. 1B). However, B19-VP1 mRNA was not detectable by the quantitative RT-PCR assay.

Antibody-dependent enhancement of B19 infection in U937 cells

To investigate the effect of IgG anti-B19 antibody at B19 infection, we added purified human IgG to the in vitro B19 infection system of U937 cells. We detected neither IgM nor IgG anti-B19 antibody activity in ET, where anti-B19 neutralizing activity was negative. But, IgG from MT, YM and TS healthy volunteers had anti-B19 antibody activity and markedly inhibited B19 proliferation, when added to KU812Ep6 cells at B19 infection. This is not the case at B19 infection of U937 cells. The incubation of U937 cells caused an enhanced increase of B19-DNA in the presence of purified human IgG from MT, YM and TS (Fig. 2A). The maximum effect of the increase on B19-DNA replication by IgG anti-B19 antibody occurred at concentration 10 μg/ml in IgG samples from MT, YM and TS. The maximum copy number of B19-DNA (1,930,000 ± 192,000 copies) with IgG from MT at this time increased more than 83 times, compared with the copy number of B19-DNA (23,000 ± 10,400 copies) without IgG (Fig. 3A). In case of IgG from YM or TS, the maximum copy number of B19-DNA (670,000 ± 163,000 and 546,000 ± 253,000 copies, respectively) increased more than 246 or 107 times, compared with the copy number of B19-DNA (2720 ± 197 and 5100 ± 232 copies, respectively) without IgG, respectively (Fig. 3A). An increased association of B19 to U937 cells in the presence of IgG anti-B19 antibody was seen from Day 0 in MT, YM and TS when compared with that in ET, which had no neutralizing activity against B19. Increased B19-DNA replication tended to be seen after Day 4 for MT, YM and TS, and the effect of the increase on B19-DNA replication continued

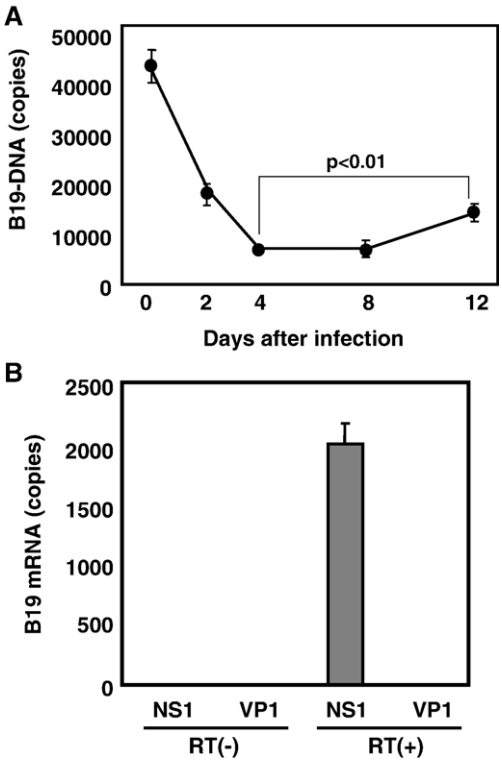

Fig. 1. B19 infection of U937 cell line. (A) B19-DNA replication in U937 cells detected by PCR. U937 cells were incubated with B19 for 0, 2, 4, 8 and 12 days. DNA was extracted at each time point, and B19-DNA was measured by PCR in triplicate. The average copy number of B19-DNA in total cell culture and standard deviation was shown. (B) Detection of B19-mRNA in U937 cells by RT-PCR. U937 cells were incubated with B19 for 6 days. RNA was extracted, and B19-RNA was measured by RT-PCR in triplicate. The average copy number of B19-DNA in proportion to 10^{10} copies of GAPDH mRNA and standard deviation are shown. RT-PCR was done by using sets of primers for NS1 and VP1 (RT(+)), and, at the same time, PCR was also done without reverse transcription to evaluate contamination of DNA (RT(−)).

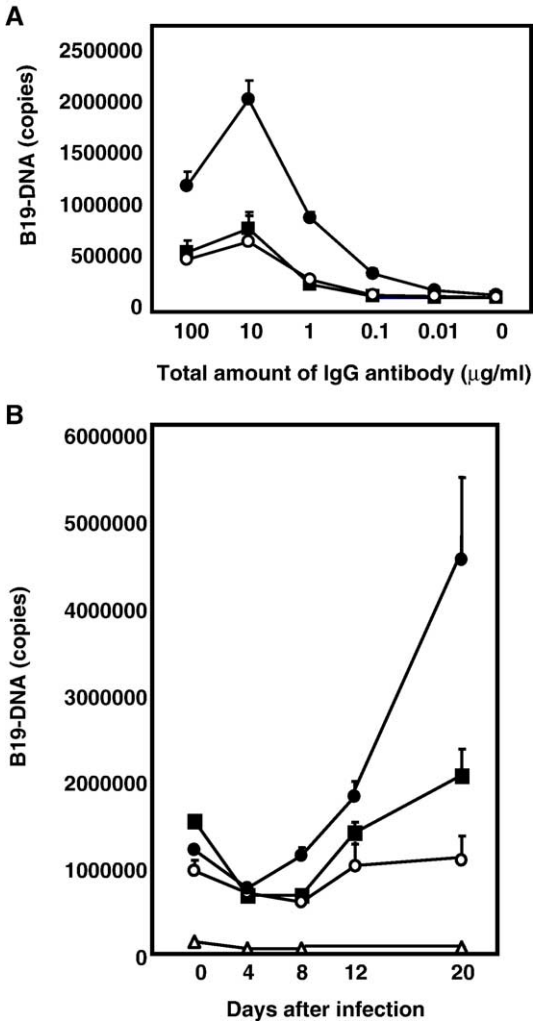

Fig. 2. B19 infection of U937 cell line in the presence of IgG anti-B19 antibodies. (A) B19-DNA detected in U937 cells in the presence of IgG anti-B19 antibodies at Day 8 of incubation. In vitro B19 infection of U937 was done in the presence of various concentrations of IgG anti-B19 antibodies from MT (●), YM (■) and TS (○) as indicated. B19-DNA was measured by PCR in triplicate. The average copy number of B19-DNA in total cell culture and standard deviation were shown. (B) B19-DNA detected in U937 cells in the presence of 10 μg/ml of IgG anti-B19 antibodies. In vitro B19 infection of U937 was done in the presence of IgG anti-B19 antibodies from MT (●), YM (■), TS (○) and *ET* (△) for 0, 4, 8, 12 and 20 days. DNA was extracted at each time point. B19-DNA was measured by PCR in triplicate. The average copy number of B19-DNA in total cell culture and standard deviation are shown.

until Day 20, the last day of our observation (Fig. 2B). The increase of B19-DNA induced by the presence of IgG anti-B19 antibody was inhibited by adding anti-Fc receptor antibodies. Increased B19-DNA by 10 μg/ml of IgG from MT at Day 8 was significantly inhibited by the presence of 5 μg/ml of anti-Fc receptor antibody, but the increased B19-DNA replication was not reduced in the presence of the same concentration of the control antibody 1F5 (Fig. 3).

Increased production of TNFα

We measured the amount of TNFα produced in the culture media of the in vitro B19 infection system of U937 by using

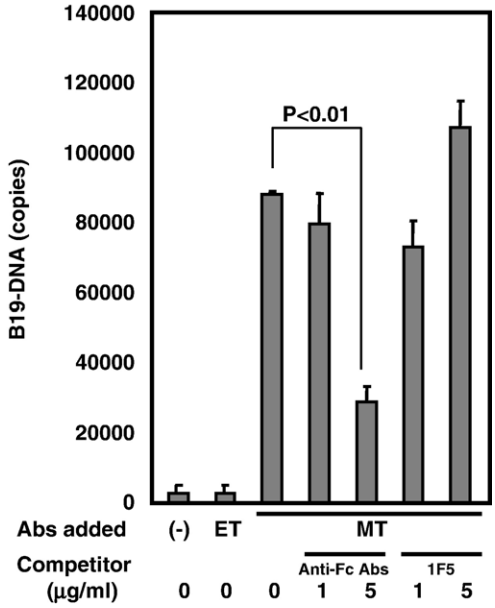

Fig. 3. Inhibition of ADE by anti-Fc antibodies. In vitro B19 infection of U937 was done in the presence or absence of 10 μg/ml of IgG anti-B19 antibodies from ET or MT. At the same time, anti-Fc antibodies or isotype-matched control antibody 1F5 were added to the culture as the competitor for ADE at 0, 1 and 5 μg/ml concentrations. DNA was extracted at Day 8. B19-DNA was measured by PCR in triplicate. The average copy number of B19-DNA in total cell culture and standard deviation are shown.

ELISA. The level of TNFα production significantly increased in the presence of IgG from MT that had reactivity against B19, but not in the presence of IgG from ET that had no reactivity against B19 (Fig. 4).

ADE was also observed in bone marrow samples

The increase of B19-DNA induced by the presence of IgG anti-B19 antibody was also observed in bone marrow samples, and increased B19-DNA was significantly inhibited by adding anti-Fc receptor antibodies like in case of U937 (Fig. 5A).

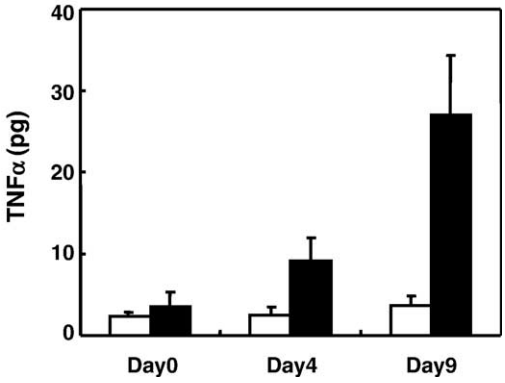

Fig. 4. Secretion of TNFα in culture supernatant of U937 infected with B19. In vitro B19 infection of U937 was done in the presence of 10 μg/ml of IgG anti-B19 antibodies from MT (■) or ET (□). The concentration of TNFα secreted in the culture supernatant was measured by ELISA in triplicate on 0, 4 and 9 days. The average amount of TNFα in total cell culture and standard deviation are shown.

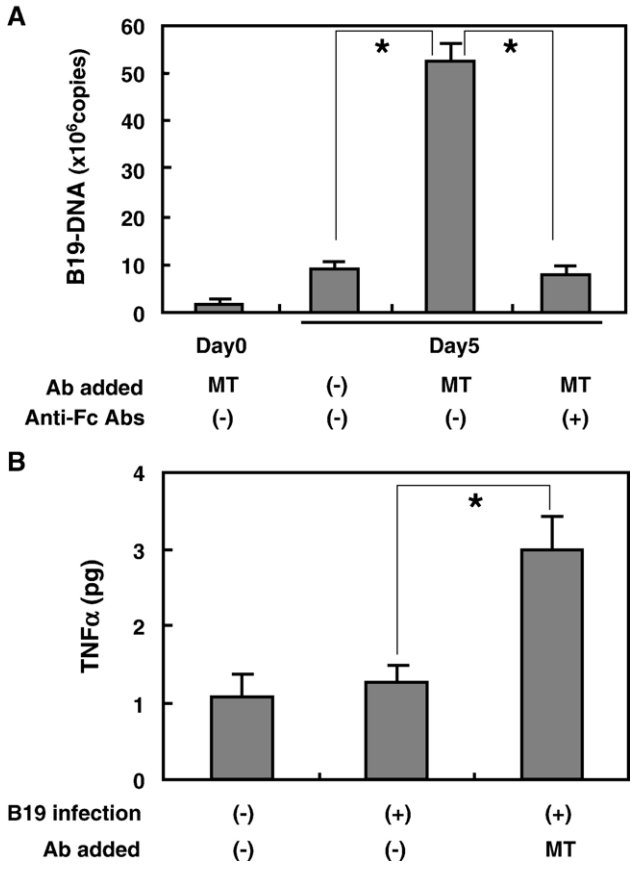

Fig. 5. ADE in B19 infection of bone marrow cells. (A) Enhanced B19-DNA replication in the presence of anti-B19 antibody and its inhibition by anti-Fc antibodies in B19 infection of bone marrow cells. In vitro B19 infection of bone marrow cells was done in the presence or absence of 10 μg/ml of IgG anti-B19 antibodies from MT. At the same time, anti-Fc antibodies were added to the culture as the competitor for ADE at 0 and 5 μg/ml concentrations. DNA was extracted at Day 5. B19-DNA was measured by PCR in triplicate. The average copy number of B19-DNA in total cell culture and standard deviation are shown. *$P < 0.01$. (B) Enhanced TNFα secretion in culture supernatant of bone marrow cells infected with B19. In vitro B19 infection of bone marrow cells was done in the presence of 10 μg/ml of IgG anti-B19 antibodies from MT. The concentration of TNFα secreted in the culture supernatant was measured by ELISA in triplicate on 0 and 5 days. The average amount of TNFα in total cell culture and standard deviation are shown. *$P < 0.05$.

Moreover, the level of TNFα production also increased significantly in the presence of IgG from MT (Fig. 5B).

Discussion

The participation of nonerythroid cells in B19 infection in vitro has not so far been described. This is attributed to the evidence that only erythroid cells expressing P antigen are permissive to B19 infection that produces the B19 protein VP1. We here demonstrated that B19 infection was involved in nonerythroid cells, especially immune cells, because: B19-DNA was detectable in the nuclear fraction of PBMC from patients with acute B19 infection (Anderson et al., 1985); monocytic cell line U937 bound B19 in vitro (Brown and Young, 1997); B19-DNA replication in U937 was slow, but the B19-DNA levels increased at Day 12 when compared with at Day 4 of B19 inoculation (Brown et al., 1993); ADE occurs at B19 infection of Fc receptor-positive cells (Cancel Tirado and Yoon, 2003).

Despite the marked association of B19 to U937 at Day 0, B19-DNA decreased at Day 4. Then, B19-DNA gradually increased after that. This indicated that the limited numbers of B19 entered into the cells. B19 infection of U937 cells, however, may be abortive because the infection is not accompanied by the production of viral particle. One mechanism that may explain the reason why B19 infection fails to cause capsid protein synthesis in U937 is that nonerythroid cells may lack cellular factors needed for B19 replication (Ray et al., 2001; Rhode and Paradiso, 1989), although we have no evidence at present. The defective translocation of NS1 protein into the nucleus may also be responsible for this mechanism as shown at fibroblasts in the case of parvovirus H-1 (H-1) in the Parvoviridae (Rhode and Paradiso, 1989). Another possibility is the effect of TNFα which is produced in B19-infected U937 (Fu et al., 2002), and this may cause an incomplete shut-off of host protein synthesis, resulting in the inefficient replication of the virus (Lopez-Guerrero et al., 1990). The production of nitric oxide and superoxide anions may also indicate abortive H-1 infection in U937 (Lopez-Guerrero et al., 1997). Thus, B19 infection failed to cause the production of B19 particle in monocytic cell line U937. However, we cannot conclude that B19 infection of macrophages is abortive in vivo because B19-VP1 and VP1 mRNA were positive in macrophages or T cells at acute B19 infection or in rheumatoid synovium (Takahashi et al., 1998). It should be here noted that, although influenza virus infection of lymphocytes is abortive in vitro, the lymphocytes can synthesize influenza virus proteins when infected with the virus in the presence of macrophages (Mock et al., 1987).

The ADE mechanism was first described for flaviviruses (Hawkes, 1964) and then for a number of other viruses, including influenza virus (Ochiai et al., 1988), dengue virus (Halstead and O'Rourke, 1977), HIV-1 (Robinson et al., 1988; Takeda et al., 1988), rabies virus (Porterfield, 1981) and the parvoviridae Aleutian mink disease parvovirus (Kanno et al., 1993). The mechanism by which ADE of virus infection is mediated is primarily through interaction of the Fc region of virus-specific IgG and Fc receptors on the surface of phagocytes (Peiris et al., 1981). IgG anti-B19 antibody from healthy volunteers also had a strong inhibitory ability for B19 infection in erythroid cell line KU812Ep6 that lacked Fc receptor on their surface (data not shown) but enhanced B19-DNA replication in macrophage cell line U937 when added at the initial phase of B19 infection. We believe that this study is the first one to show ADE in human parvovirus B19 infection. Like other types of virus infection, ADE in B19 infection was mediated by the Fc receptor on the cell surface. Of importance is that ADE may also occur in vivo and may associate with the life-threatening dengue hemorrhagic fever and dengue shock syndrome (Halstead, 1988). Many studies also have indicated the role of ADE in persistent infection of viruses (Cancel Tirado and Yoon, 2003). It is actually plausible to speculate that ADE in B19 infection occurs in vivo since the infection to the bone marrow cells of B19 is enhanced in the presence of IgG anti-B19

antibody (MT), and more B19-DNA came to be detected. The production of TNFα was also enhanced, although quantity of production was lower than that observed in U937. This may be due to the heterogeneity of bone marrow cells. As the bone marrow cells consist of variety lineage cells, B19 may infect to cells that have no capability of TNFα production.

It is worth to discuss here the presented data in association with a variety of clinical observation that are difficult to explain solely by the mechanism for the apoptosis induction in B19-infected erythroid lineage cells at acute B19 infection (Moffatt et al., 1998; Ozawa et al., 1988). One of the clinical features of B19 infection is prolonged or persistent infection where autoantibody production, autoimmune disease-like signs or the production of inflammatory cytokines such as TNFα and interferon-gamma is often accompanied, indicating the role of immune system at B19 infection. This paper demonstrates the role of B19 infection in immune abnormality in macrophage cell line U937, where TNFα is markedly secreted at ADE mechanism. It is, therefore, reasonable to speculate that infection of B19 in immune cells may associate with a variety of immune abnormalities that associate with pathogenesis of rheumatoid arthritis (Takahashi et al., 1998).

Materials and methods

Cells

Human monocytic cell line U937 was provided by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. Human erythroid cell line KU812Ep6 was kindly provided by E. Miyagawa at the Institute of Fuji Rebio Inc (Tokyo, Japan) (Miyagawa et al., 1999). The U937 cell lines were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS) and 50 μg (each) of streptomycin and penicillin (Gibco BRL, Carlsbad, CA) per milliliter in a 5% CO_2 atmosphere at 37 °C. KU812Ep6 cells were cultured with the same medium containing 6 IU/ml of erythropoietin (Kirin Brewery Inc., Tokyo, Japan). Bone marrow samples were obtained from the volunteers who gave informed consent for the use of their samples for our study.

Human parvovirus B19

Serum from patient FU with acute B19 infection was used for in vitro infection assay. The serum contained 2.5×10^{14} copies of B19-DNA per milliliter, and anti-B19 antibodies were not detected by using enzyme-linked immunosorbent assay (ELISA).

Human IgG samples

Human IgG was purified from serum of each normal volunteer (MO, ET, MT, YM and TS) by using MAbTrapG2 (Amersham Pharmacia Biotech, Piscataway, NJ). The titers of anti-B19 IgM and anti-B19 IgG were measured by using a commercial indirect ELISA kit (Denka Seiken Inc., Tokyo, Japan). The titer of anti-B19 antibodies in the original serum was expressed as an index according to the instructions provided, and an index number over 0.8 was positive for the presence of anti-B19 antibodies. The titers of anti-B19 IgM were below 0.8 in all samples tested. The titers of anti-B19 IgG in MO, ET, MT, YM and TS were <0.8, <0.8, 10.2, 9.8 and 9.5, respectively. The neutralizing activity for B19 infection in the original serum was measured as described previously (Saito et al., 2003). There is no neutralizing activity in MO and ET. The neutralizing activity of MT, YM and TS was 93%, 60% and 55%, respectively.

Pronase treatment of PBMC

Peripheral blood mononuclear cells (PBMC) were obtained from patients with acute B19 infection by density gradient centrifugation on Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden). Cells were suspended in PBS at concentration 5×10^6/ml, and 0.25 mg/ml of pronase was added. Cells were incubated at 37 °C for 60 min, and then an equal volume of pre-chilled 10% FBS-RPMI was added to stop the pronase reaction. The cells were washed with PBS three times, and then they were underwent DNA extraction.

DNA and RNA extraction

DNA was prepared from cells by using a conventional phenol-chloroform method. RNA was extracted from cells by using the acid guanidium-phenol chloroform method and using Isogen-LS (Nippon Gene, Toyama, Japan) twice. To remove DNA, 1 unit/ml of DNase (Ambion, Austin, TX) was added to the RNA suspension.

Quantitative PCR assay

A quantitative PCR assay of samples amplified the VP1-region of B19-DNA as described previously (Saito et al., 2003). The primers and probe used to detect VP1 were: forward primer, 5′-CCCTAGAAAACCCATCCTCTGTG-3′; reverse primer, 5′-AGGTTCTGCATGACTGCTACTGG-3′; probe, 5′-TCATGGACAGTTATCTGACCACCCCCA-3′. A quantitative PCR assay to detect the NS1 region of B19-DNA was done by using the same procedure and replacing the PCR primers and probe. The primers and probe used to detect NS1 were: forward primer, 5′-AACCCCGCGCTCTAGTACG-3′; reverse primer, 5′-CTGGGCTTCCGACAAATGA-3′; probe, 5′-CATCCCCGGGACCAGTTCAGG-3′.

Quantitative RT-PCR assay

Total RNA samples (1 μg) were subjected to quantitation of B19-NS1 mRNA, B19-VP1 mRNA and human glyceraldehydes-3-phosphate dehydrogenese (GAPDH) mRNA. The quantity of each mRNA was measured by using a method based on the TaqMan protocol (Applied Biosystem, Foster City, CA). Reverse transcription and PCR (RT-PCR) was done in a one-step reaction as described previously (Fu et al., 2002).

Cell culture for in vitro B19 infection

Human monocytic cell line U937 (4×10^6 cells/ml) was mixed with human serum FU that contained 2.5×10^{14} copies of B19-DNA/ml from a patient with acute B19 infection. The final concentration of the B19 serum FU was 1:2500. For the experiment of human IgG *MT* addition, B19 serum FU was appropriately diluted with 10% FBS-RPMI medium containing human IgG *MT* and was incubated at 37 °C for 1 h before the cell culture. The cells were incubated at 37 °C in 5% CO_2 for 12 h. The cells were washed three times with PBS and were adjusted to 5×10^5 cells/ml and were further cultured with 10% FBS-RPMI medium at 37 °C in 5% CO_2. For the blocking experiment of Fc receptor, U937 cells were incubated for 1 h with 0.5 μg/ml or 2.5 μg/ml of anti-CD32 and anti-CD64 monoclonal antibodies (PharMingen, San Diego, CA) at 4 °C, were washed three times with PBS and then were used for the B19 infection experiment described above.

Human bone marrow cells (1×10^5/ml) were incubated with B19 serum FU in the presence or absence of human IgG MT at same manner described above and cultured with 10% FBS-RPMI medium at 37 °C in 5% CO_2 for 5 days. Then, they were subjected for DNA extraction to evaluate quantity of B19-DNA by PCR. Culture supernatant was collected at Day 5 and measured the concentration of TNFα.

ELISA for measuring TNFα

The amount of human TNFα in the culture supernatant was measured by using an ultrasensitive ELISA kit (BioSource International, Camarillo, CA) in triplicate. The minimum detectable dose of TNFα was <0.09 pg/ml, according to the manufacturer's instructions.

Acknowledgments

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